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REVIEW ARTICLE

Emerging treatments for chronic hepatitis C

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Advances in understanding the hepatitis C virus (HCV) life cycle and the urgent need to find complementary direct-acting antiviral (DAA) therapies has led to substantial advancements in treating chronic hepatitis C. The introduction of telaprevir and boceprevir in 2011 increased the sustained virological response (SVR) rate from approximately 50% to > 70%, but this therapy further restricted patient eligibility and is only approved for treating HCV genotype 1 infection. Interferon has long remained the backbone of HCV therapy and helps prevent viral breakthrough. However, interferon has limited effectiveness and is associated with severe adverse effects and toxicity, especially among cirrhotic patients. Moving to interferon-free therapies should greatly improve SVR rates and offer new treatments for other HCV genotypes and for ineligible patients or patients failing to respond to prior therapies. However, without the relative safety of interferon to suppress viral escape, vigilance will be required to select appropriate therapies and monitor resistance. Several DAAs are currently undergoing clinical trials and will soon undergo the approval process. Goals of future HCV clinical research will be to identify combinations of DAAs with high genetic barriers, investigate optimal treatment doses and durations, and determine the role of ribavirin in DAA therapies.

Introduction

At least 185 million people may be chronically infected with hepatitis C virus (HCV).[1,2] These individuals should be treated to prevent the greatly increased lifetime risk of cirrhosis, liver failure, and hepatocellular carcinoma. Successful HCV therapy eradicates the virus, defined as a sustained virological response (SVR) in which the virus remains undetectable 24 weeks after the end of treatment. Until recently however the success rate of the standard of care—peginterferon plus ribavirin combination

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therapy—was < 50% in patients with genotype 1 infection, despite a daunting 48 week treatment regimen requiring weekly interferon injections.[3–5]

Unsuccessful treatment can be classified as a "null response" when the virus remains detectable throughout treatment. "Viral breakthrough" is when the virus becomes transiently undetectable but rebounds before the end of treatment. "Relapse" is when the virus becomes transiently undetectable but then reappears after the end of therapy. In 2011, the approval of the direct-acting antiviral (DAA) drugs telaprevir (VX-950/MP-424) and boceprevir increased the sustained virological rate to 70—80%, depending on genetic factors and previous treatment history.[6,7] Despite these gains, these first-generation protease inhibitors will soon face obsolescence as new DAAs progress through clinical trials.

Molecular insight into the HCV life cycle gained through advances in cell culture and small animal models has facilitated the development of DAAs, which are small molecule therapies designed to directly target viral products at various steps in the viral life cycle. All HCV products appear to be required for replication and drugs that target each viral product are under investigation (Fig. 1). This DAA approach promises high specificity and fewer adverse effects, although these benefits are offset by high costs and a higher risk of resistance. The problem of resistance can be partly addressed by the coadministration of several DAAs that target different viral products to create a higher barrier to resistance. The problem of cost may be managed partly by careful patient selection. To overcome the limitations of telaprevir and boceprevir, priorities for future DAA development include improved potency; improved genotypic coverage, especially against genotype 4; a higher barrier to resistance; and better safety profiles.[8] Secondary priorities include shorter treatment duration, better patient compliance through reduced pill burden, minimized drug—drug interactions, and lower cost.

A major goal of HCV research is the development of safe and effective short duration all-oral, interferon-free therapies. Interferon is poorly tolerated, especially among elderly and cirrhotic patients, and is associated with fever, tachycardia, headache, joint or muscle pain, fatigue, skin lesions, depression, and neuropsychiatric effects.[9,10] Compared to other chronic diseases such as human immunodeficiency virus (HIV), the drug development time for HCV is fortunately benefitted by a short treatment duration and by the use of open-label studies and alternative end points such as SVR12 (i.e., the SVR at 12 weeks is the end point instead of 24 weeks). By contrast, HCV clinical trials are complicated by inter- and intragenotype variability; prior patient treatment history; patients with cirrhosis or liver transplantation; HIV or hepatitis B virus (HBV) coinfection; and a high risk of resistance, unless accompanied by peginterferon and ribavirin or in combination with other DAAs. Clinical trials often initially exclude such patients; however, these patients are often the ones who will benefit the most from interferon-free therapies. Recent studies have increasingly begun to focus on some of these heterogeneous and difficult-to-treat patient subgroups.

NS3/4A protease inhibitors

Direct-acting antivirals act by specifically targeting viral components. Telaprevir, one of the first United States Food and Drug Administration (US FDA)-approved protease inhibitors (PIs), mimics the carboxy-terminal region of the HCV NS3/4 serine protease.[11] The 9.6 kb HCV RNA genome contains a single open reading frame and encodes a single approximately 3000 amino acid polyprotein that must be cleaved into three structural proteins, which includes the core protein, two envelope proteins, and six nonstructural proteins. Cellular proteases cleave the structural proteins, whereas virally encoded proteases NS2 and NS3, and the NS3 cofactor NS4A, cleave the remaining polyprotein at four specific sites to produce the nonstructural proteins. Because NS3/4A also interferes with innate immune activity by degrading key immune signaling molecules, targeting NS3/4A prevents cleavage of the nonstructural proteins and inhibits the ability of the virus to evade the immune response.[12–14]

Boceprevir triple therapy

Several studies have demonstrated the increased effectiveness of boceprevir or telaprevir triple therapy, compared to the standard of care with peginterferon and ribavirin combination therapy.[15] Boceprevir requires a

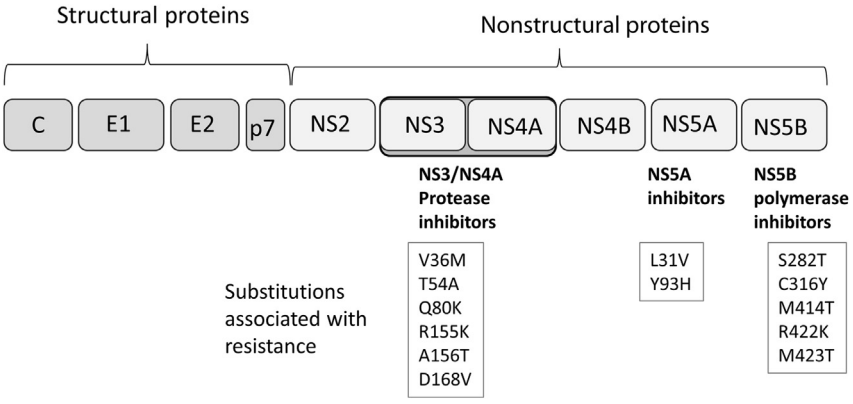

Figure 1 The hepatitis C virus (HCV) genome architecture. The HCV RNA genome is initially translated as a polyprotein. Host proteases cleave the structural core and envelope proteins, whereas the viral encoded NS3/NS4A protease cleaves itself and the other nonstructural proteins. Clinically relevant resistance mutations have been detected for each class of direct-acting antiviral.

"lead-in" phase of peginterferon and ribavirin alone for 4 weeks, which is intended to lower HCV RNA levels and reduce the risk of viral breakthrough resulting from the selection for boceprevir-resistant strains.[16] The Serine Protease Inhibitor Therapy 2 (SPRINT-2) trial and the Retreatment with HCV Serine Protease Inhibitor Boceprevir and PegIntron/Rebetol 2 (RESPOND-2) trial assessed the effect of triple therapy with boceprevir plus peginterferon and ribavirin in 1097 treatment-naïve patients and 403 previously treated patients, respectively. In both studies, patients received peginterferon plus ribavirin for 4 weeks (i.e., the lead-in period), after which they were assigned to one of three groups: (Group 1, the control group) peginterferon, ribavirin, and placebo for 44 weeks; (Group 2) peginterferon, ribavirin, and boceprevir for 24 weeks (SPRINT-2) or 32 weeks (RESPOND-2), and an additional 20 weeks (SPRINT-2) or 12 weeks (RESPOND-2) if HCV RNA remained detectable; or (Group 3) peginterferon, ribavirin, and boceprevir for 44 weeks. In the SPRINT-2 study, 40% of patients in Group 1, 67% of patients in Group 2, and 68% of patients in Group 3 achieved SVR; 21% of boceprevir-treated patients underwent anemia-related dose reductions.[17] In the RESPOND-2 study, only 21% of patients in the control group achieved SVR, compared to 59% of patients in Group 2 and 66% of patients in Group 3, although anemia occurred frequently in the boceprevir-treated patients and was treated with erythropoietin.[18]

Telaprevir triple therapy

A major difference between telaprevir and boceprevir is that a lead-in phase is not required with telaprevir triple therapy because of its stronger anti-HCV potency. The Phase III (ADVANCE) study reported SVR rates of 75% after 12 weeks of telaprevir, peginterferon, and ribavirin triple therapy and 69% after 8 weeks, compared to 44% for peginterferon and ribavirin alone in treatment-naïve patients[6]; however, adverse events such as pruritis, rash, and nausea were higher in the telaprevir treatment arms. In the ILLUMINATE noninferiority study examining treatment duration, 540 treatment-naïve patients were treated with telaprevir triple therapy for 12 weeks, which was followed by peginterferon and ribavirin alone for an additional 4 weeks (T12PR24) or 28 weeks (T12PR48), depending on whether the patient had an extended rapid virological response (i.e., undetectable HCV RNA at Week 4 and Week 12).[19] Ninety-two percent of patients in the T12PR24 group and 88% of patients in the T12PR48 group achieved SVR, although rash and anemia were common and sometimes severe, which led to discontinuation in 18% of patients. The REALIZE study demonstrated substantial improvements in SVR rates among previously treated patients: 64% SVR rate after 12 weeks of telaprevir triple therapy, 66% SVR rate when preceded by a 4-week lead-inphase, and 17% SVR rate after peginterferon and ribavirin alone.[7] Based on the strong improvement among prior relapsers, the FDA approved response-guided therapy with early termination after 24 weeks for prior relapsers who maintained undetectable HCV RNA at Week 4 and Week 12 (i.e., extended rapid virological response).[20] A Phase III study in Japan reported an SVR rate of 73% after 12 weeks of triple therapy that was followed by 12 weeks of combination therapy, compared to an SVR rate of 49% among patients assigned to 48 weeks of combination therapy.[21] However, adverse effects such as anemia and skin disorders were approximately twice as common among patients treated with triple therapy (38% and 47%, respectively) than among patients treated with combination therapy (18% and 24%, respectively). Another Japanese Phase III study reported SVR rates of 88% among prior relapsers and 34% among prior nonresponders, but most patients experienced skin disorders (82%) and nearly all patients required ribavirin dose reduction (99%).[22] Twenty-one percent of patients discontinued telaprevir only, and 16% of patients discontinued all drugs. Because the dosage of telaprevir is not adjusted by body weight, anemia is of particular concern in Japan because of the larger proportion of older female patients and the typically lower body weight of Japanese patients, compared to patients in many Western countries.[23]

Adverse events associated with telaprevir and boceprevir triple therapy

The addition of boceprevir or telaprevir to a treatment regimen containing peginterferon and ribavirin leads to a higher frequency of adverse events, although many adverse events reported in clinical trials are also those associated with peginterferon and ribavirin treatment. Boceprevir triple therapy is associated with increased risk of neutropenia, dysgeusia, anemia, and thrombocytopenia, compared to peginterferon plus ribavirin dual therapy.[15,24] In clinical trials, approximately 33% of patients experienced neutropenia and 25% of patients experienced anemia, although the frequency of discontinuation was not greater. Telaprevir triple therapy is associated with increased frequency of anemia, pruritis, and rash in up to 50% of patients, and associated with severe events in up to 5—10% of patients.[15,24] Anorectal adverse events and rates of discontinuation are higher among patients treated with telaprevir triple therapy than among patients treated with peginterferon and ribavirin alone.

Telaprevir and boceprevir in cirrhotic patients

The risk of adverse events is particularly important in the treatment of cirrhotic patients. In the Compassionate Use of Protease Inhibitors in Viral C Cirrhosis (CUPIC) study, previously treated patients with compensated cirrhosis were treated for 48 weeks with peginterferon and ribavirin and either boceprevir or telaprevir.[25] Depending on the response to previous therapy, patients achieved a SVR rate of 19—74% with telaprevir and a rate of 0—54% with boceprevir, but severe adverse events occurred in 50% of patients.

Telaprevir and antiviral resistance

Telaprevir triple therapy substantially improves the SVR rate over peginterferon plus ribavirin, especially among treatment-experienced patients; however, if administered alone, telaprevir induces rapid selection for resistance variants.[26,27] Therefore, telaprevir must be administered as part of triple therapy with peginterferon and ribavirin to

suppress viral breakthrough. Triple therapy consequently only increases the risk of adverse effects and imposes further restrictions on an already restrictive therapy. Because of substantial intergenotypic variation in the NS3 domain, the high specificity of first-generation PIs limits their application to genotype 1 infections and results in a low barrier to resistance.[28] Even within genotype 1, resistance occurs more frequently in genotype 1a than in genotype 1b because of a synonymous codon at R155 that reduces the number of nucleotide changes required to cause an amino acid substitution.[29] Resistance mutations tend to compromise viral fitness, but compensatory mutations such as V36 M that restore viral fitness often occur, which allows the virus to compete more effectively with the wild type virus, even in the absence of the drug.[30] High cross-resistance unfortunately also implies that resistance to one PI is likely to confer resistance to other PIs in the same class. Discontinuing telaprevir or boceprevir therapy is consequently recommended in the event of viral breakthrough. Triple therapy also involves a heavy pill burden with weekly injections of peginterferon, twice-daily ribavirin intake, and twice (q12) or three times (q8) daily telaprevir or boceprevir intake. Telaprevir must also be taken after a high fat meal (20 g) such as a bagel with cream cheese or cup of ice cream, which imposes inconvenient lifestyle changes and risks poor patient compliance.

Second-wave protease inhibitors

Second-wave PIs (Table 1) attempt to address these limitations through an incremental approach to increase the barrier to resistance and to improve activity against other genotypes, especially the common but difficult-to-treat genotype 4 for which there are currently few treatment options. Second-wave PIs also aim to improve patient compliance and tolerability by reducing the dosing schedule to one or two administrations per day and by improving the safety profile.[31] Second-generation PIs attempt to go a step further and provide pangenotypic activity against all HCV genotypes and resistance mutations that affect the first-generation PIs.

Simeprevir triple therapy

Simeprevir (TMC-435) is a once-daily macrocyclic PI with a long half-life that is active against genotypes 1, 2, 4, 5, and 6.[32] Simeprevir has been approved in the United States of America (150 mg dose) and in Japan (100 mg dose) for use in triple therapy with peginterferon and ribavirin. In the Phase III QUEST-1 clinical trial, 394 treatment-naïve patients with genotype one in 13 countries were randomized to treatment with peginterferon-α2a plus ribavirin and either simeprevir or a placebo for 12 weeks, followed by response-guided therapy for 24 weeks or 48 weeks.[33] Eighty percent of patients in the simeprevir group achieved SVR12, compared to 50% in the placebo group. Fatigue and headache were the most common adverse events but these events occurred at similar frequencies in the simeprevir group and in the placebo group. The QUEST-2 study design was similar, but it included treatment arms to compare peginterferon-α2a versus peginterferon-α2b and two ribavirin dosages.[34] Eighty-one percent of patients in the

Table 1 NS3/4A protease inhibitors undergoing development.

NS3/4A protease inhibitors	Manufacturer	Status	Structure	Barrier	Adverse effects	Gt
First-generation, first-wave						
Telaprevir (VX-950, Incivek)	Janssen	Approved	Linear (covalent)	Low	rash, anemia, pruritis	1
Boceprevir (SCH503034, Victrelis)	Merck	Approved	Linear (covalent)	Low	anemia, dysgeusia	1
First-generation, second-wave						
Simeprevir (TMC-435, Olysio)	Tibotec	Approved	MacrocycLic	Moderate	anemia, bilirubin	1,2,5,6
Faldaprevir (BI-201335)	Boehringer Ingelheim	Phase III	Linear	Moderate	rash, jaundice, nausea, diarrhea	1
Asunaprevir (BMS-650032)	Bristol–Myers Squibb	Pending approval	Linear	Moderate	PR	1,4
ABT-450/r	Abbvie	Phase III	Linear	Moderate	fatigue, headache, nausea	1
Danoprevir (ITMN-191, RG 7227)	Roche	Phase II	MacrocycLic	High	nausea, diarrhea, neutropenia, ALT	1,2,4
Sovaprevir (ACH-1625)	Achillion	Phase II	Linear	Moderate	PR	1
Vedroprevir (GS-9451)	Gilead	Phase II	MacrocycLic	Moderate	PR	1
IDX320	Idenix	Phase II	MacrocycLic	Moderate	PR	1,2,3,4
Vaniprevir (MK-7009)	Merck	Phase III	MacrocycLic	Moderate	nausea	1
Second-generation						
MK-5172	Merck	Phase III	MacrocycLic	Moderate	PR	1,2,4,5,6
ACH-2684	Achillion	Phase II	MacrocycLic	High	PR	1,2,4,5,6

ALT = alanine aminotransferase; Barrier = genetic barrier to resistance; Gt = genotype; PR = adverse effects are similar to those of peginterferon plus ribavirin combination therapy.

simeprevir group achieved SVR12, compared to 50% in the placebo group. The incidence of anemia was similar in both groups, but rash and photosensitivity were more common in the simeprevir group. The Phase III PROMISE study examined the effect of 12 weeks of peginterferon-α2a plus ribavirin and either simeprevir or placebo, followed by response-guided therapy in prior relapsers.[35] The SVR12 rate was 79% in the simeprevir group and 36% in the placebo group. Both groups had a similar incidence and severity of adverse events.

In the CONCERTO-1 Phase III trial in Japan, 183 treatment-naïve patients were randomized to simeprevir, peginterferon, and ribavirin for 12 weeks. This was followed by response-guided therapy for 12 weeks or 36 weeks or by a placebo for 12 weeks, followed by 36 weeks with peginterferon and ribavirin.[36] Sustained virological response at 12 weeks was achieved in 89% of simeprevir-treated patients, compared to 62% of placebo-treated patients. In the CONCERTO-2 and CONCERTO-3 open-label Phase III trials, 155 prior nonresponders and relapsers were treated with simeprevir, peginterferon, and ribavirin for 12 weeks or 24 weeks, followed by response-guided therapy. Fifty-three percent of prior nonresponders and 96% of prior responders achieved SVR12, but 13% of prior nonresponders experienced viral breakthrough and 39% experienced viral relapse.[37] In a Phase IIb, randomized, double-blind, placebo-controlled trial (NCT00980330), 462 genotype 1 prior relapsers and nonresponders were treated with peginterferon, ribavirin, and simeprevir or a placebo for 12 weeks, 24 weeks, or 48 weeks.[38] The SVR rates were significantly higher in the simeprevir treatment arms, regardless of prior treatment response, and up to 89% among prior relapsers.

Polymerase inhibitors

The PIs act during the early stage of the HCV life cycle by interfering with polyprotein cleavage, whereas other DAAs target different aspects of viral replication. Polymerase inhibitors target HCV NS5B RNA-dependent RNA polymerase (RdRp/NS5B; Table 2). This low-fidelity polymerase synthesizes a negative strand RNA, which is then used to produce multiple positive strand copies of the HCV genome for replication and translation.[39] Two different types of polymerase inhibitors—nucleoside inhibitors (NIs) and non-nucleoside inhibitors (NNIs)—are undergoing clinical trials.

Nucleoside inhibitors are similar to naturally occurring nucleotides; however, when they are incorporated into the elongating RNA sequence, they inhibit the RdRp active site and cause chain termination.[40] An NI is administered as a prodrug and must be phosphorylated to become an active nucleoside triphosphate. In principle, NIs have a low barrier to resistance because of the potential for single amino substitutions to confer resistance. In practical terms, an effective barrier to resistance is relatively high, owing to strong conservation of the active site among all HCV genotypes and the poor fitness of resistant variants.[40] In fact, the NIs have the highest barrier to resistance among DAAs examined to date.[31]

The NIs directly interfere with the RdRp active site, whereas the NNIs suppress RdRp activity by binding to allosteric sites away from the active site. The NNIs are only active against genotype 1 and have lower antiviral potency and a lower barrier to resistance. Resistance mutations also do not necessarily compromise viral fitness. However, several NNIs under investigation, such as the thumb I/II inhibitors and palm I/II inhibitors, may provide complementary protection by targeting different regions of the molecule.

Sofosbuvir, a nucleoside polymerase inhibitor, was approved in the United States for use in combination with ribavirin to treat genotypes 2 and 3 and for use in combination with peginterferon and ribavirin to treat genotypes 1 and 4. The safety and efficacy of sofosbuvir was evaluated in a Phase II clinical trial in which 122 treatment-naïve genotype 1 patients were randomly assigned to 200 mg or 400 mg of sofosbuvir plus peginterferon and ribavirin for 12 weeks, followed by 12 weeks or 36 weeks of

Table 2 NS5B polymerase inhibitors undergoing development.

Type	Polymerase inhibitors	Manufacturer	Status	Genotype
Nucleoside inhibitors				
	Sofosbuvir (GS-7977)	Gilead Sciences	Approved	1–6
	Mericitabine (RG-7218)	Roche	Phase III	1,4
Non-nucleoside inhibitors				
Thumb I inhibitors				
	Deleobuvir (BI 207127)	Boehringer Ingelheim	Phase II	1
Thumb II inhibitors				
	GS-9669	Gilead Sciences	Phase I	
	Filibuvir (PF-868554)	Pfizer	Phase II	1
	VX-222	Vertex	Phase II	1
	BMS-791325	Bristol-Myers Squibb	Phase III	
Palm I inhibitors				
	Dasabuvir (ABT-333)	Abbott	Pending approval	1
	ABT-072	Abott	Phase II	1
	Setrobuvir (ANA-598)	Roche	Phase II	1
Palm II inhibitors				
	Tegobuvir (GS-9190)	Gilead Sciences	Phase II	1

response-guided peginterferon and ribavirin therapy. The SVR12 rates were 90% in the 200 mg group, 91% in the 400 mg group, and 58% in the placebo group. Adverse events such as fatigue, headache, nausea, and chills were consistent with those of peginterferon and ribavirin; in addition, discontinuation because of adverse events was similar among the groups.[41]

In the open-label Phase II Sofosbuvir with Pegylated Interferon alfa-2a and Ribavirin for Treatment-naive Patients with Hepatitis C Genotype-1 Infection (ATOMIC) study, 316 treatment-naïve patients with genotype 1, 4, or 6 were randomized to 12 weeks or 24 weeks of sofosbuvir plus peginterferon and ribavirin or to 12 weeks of triple therapy that was followed by 12 weeks of sofosbuvir with or without ribavirin.[42] In all treatment arms, SVR12 rates of 87–89% were achieved among genotype 1 patients, and there was no benefit in extending sofosbuvir treatment beyond 12 weeks. In the single-arm, open-label Phase III NEUTRINO study, 327 treatment-naïve patients with genotype 1 or genotype 4 were treated with 12 weeks of sofosbuvir plus peginterferon and ribavirin, which resulted in a SVR12 rate of 90%.[43] In a noninferiority trial, 499 patients with genotype 2 or genotype 3 were randomly assigned to receive 12 weeks of either sofosbuvir and ribavirin or peginterferon and ribavirin, which resulted in a SVR12 rate of 67% in both groups.

NS5A inhibitors

The HCV polyprotein is post-translationally processed into 10 viral proteins. The E1, E2, and core structural proteins are incorporated into the virus particle, whereas the nonstructural proteins NS3, NS4A, NS5A, and NS5B are essential components of the replication complex,[44] which is an endoplasmic reticulum-associated membrane structure essential for replication of the HCV genome. Each HCV protein is required for replication, whereas most drug discovery efforts have focused on the enzymes NS3 and NS5B; however, NS5A has no known enzymatic activity.[45] The successful interferon-free combination therapies require multiple complementary targets to raise the barrier to resistance. The protein NS5A is a promising target because of its pleiotropic roles in establishing the replication complex, in viral assembly, and in inhibiting apoptosis; however, the lack of enzymatic activity has complicated drug development efforts. The mechanism by which NS5A regulates replication is unclear, but the protein binds to RNA and interacts directly with NS5B RdRp, which catalyzes negative strand synthesis.[46] NS5A also interacts with the host protein cyclophilin A, and recruits apolipoprotein E. Cell-based replicon screening identified several anti-HCV compounds that act through NS5A.[45] These drugs have demonstrated high specificity and potency at picomolar concentrations, and have pangenomic activity and a high barrier to resistance (Table 3). The mechanism of NS5A inhibitors is unknown but may involve multiple effects such as inhibiting hyperphosphorylation, which appears to be required for viral replication.[47] Several NS5A inhibitors bind to NS5A domain 1 and prevent RNA binding without affecting NS5A dimerization.[48] Even though NS5A inhibitors are among the most potent antiviral molecules known, resistance variants are likely to pre-exist at low frequencies in patients prior to exposure because of the quasi-species nature of the virus, and may be rapidly selected after the administration of an NS5A inhibitor. In the event of viral breakthrough, the viral titer rebounds to pretreatment levels with few effects on fitness.[47] Therefore, NS5A inhibitors should be used in combination with drugs with nonoverlapping resistance profiles.

Table 3 NS5A inhibitors undergoing development.

NS5A inhibitors	Manufacturer	Status
First-generation		
Daclatasvir (BMS-790052)	Bristol–Myers Squibb	Phase III
Ledipasvir (GS-5885)	Gilead Sciences	Phase III
Ombitasvir (ABT-267)	AbbVie	Phase III
PPI-668	Presidio Pharmaceuticals	Phase II
PPI-461	Presidio Pharmaceuticals	Phase II
ACH-2928	Achillion	Phase II
GSK-2336805	GlaxoSmithKline	Phase II
BMS-824393	Bristol–Myers Squibb	Phase II
Samatasvir (IDX719)	Idenix Pharmaceuticals	Phase II
Second generation		
MK-8742	Merck	Phase II
ACH-3102	Achillion	Phase II
GS-5816	Gilead Sciences	Phase II

Interferon-free combination therapies

Interferon has long formed the backbone of HCV therapy, but interferon therapy requires difficult long term treatment and is associated with adverse effects that prevent some patients from receiving treatment, particularly among cirrhotic patients for whom interferon is toxic. Several common human and viral genetic polymorphisms also affect interferon sensitivity, and thereby reduce the effectiveness of therapy in affected patients. However, the inclusion of interferon with DAAs helps to prevent the emergence of resistance variants under the strong selective pressure of the drug. Because resistance is likely to arise in monotherapy involving any DAA, interferon-free trials are designed as combinations of two or more DAAs that together confer a high overall barrier to resistance (Table 4).[50–59,61–70] This often requires joint examination of two investigational drugs. Most current trials continue to use ribavirin because of its oral delivery and effectiveness in suppressing resistance, but avoiding ribavirin-induced anemia is another goal of HCV therapy.

Asunaprevir and daclatasvir

One of the first reported interferon-free therapies was the combination of the protease inhibitor asunaprevir with the NS5A inhibitor daclatasvir.[49,50] In an open-label Phase III study (NCT01497834), 222 interferon-intolerant patients or prior nonresponders with genotype 1 were treated with

Table 4 Interferon-free combination therapies.

Study	Trial	Patients	Weeks	Response
Asunaprevir and daclatasvir				
Kumada et al, 2014[51]	Phase III	222 gt1 IFN-intolerant or prior nonresponders	24	SVR24: 87% IFN-intolerant 81% prior nonresponders
Lok et al, 2014[50]	Phase IIa	101 gt1b	24	SVR12: 78%
Manns et al, 2014[52]	Phase III	745 diverse gt1b	24	SVR12: 85% (90% tx-naïve patients)
Asunaprevir, daclatasvir, and BMS-791325				
Everson et al, 2014[53]	Phase IIa	66 noncirrhotic, tx-naïve gt1	12 or 24	SVR12: 92%
Daclatasvir and sofosbuvir				
Sulkowski et al, 2014[54]	Open-label	211 patients gt1, gt2, or gt3	12 or 24	SVR12: gt1, 98%; gt2, 92%; gt3, 89%
Ledipasvir and sofosbuvir				
Afdhal et al, 2014[55,56]	Phase III	865 gt1 tx-naïve; 440 gt1 prior nonresponders	12	SVR12: tx-naïve, 96%; prior nonresponders, 94%
Kowdley et al, 2014[57]	Phase III	647 gt1, tx-naïve, noncirrhotic	8	SVR12: 94%
Sofosbuvir and ribavirin				
Jacobson et al, 2013[58]	Phase III	278 gt2/3, IFN-ineligible; 201 previously treated	12	SVR12: IFN-ineligible, 78%; previously treated, 50%
Zeuzem et al, 2014[59]	Unblinded	21 gt2, 328 gt3 cirrhotic or previously treated	12	SVR12: gt2, 93%; gt3, 85%
ABT-450/r, dasabuvir (ABT-333), and ribavirin				
Poordad et al, 2013[61]	Phase IIa	50 gt1, tx-naïve or prior nonresponder	12	SVR12: tx-naïve, 95%; prior nonresponders, 47%
ABT-450/r, ombitasvir, dasabuvir, and ribavirin				
Feld et al, 2014[62]	Phase III	631 gt1, tx-naïve	12	SVR12: gt1a, 95%; gt1b, 98%
Ferenci et al, 2014[63]	Phase III	419 gt1a, 305 gt1b	12	SVR12: gt1b, 99.5%; gt1a, 97%
Zeuzem et al, 2014[64]	Phase III	394 gt1, prior nonresponders	12	SVR12: 96%
Poordad et al, 2014[65]	Phase III	380 gt1 Child-Pugh A cirrhotics	12 or 24	SVR: 12 weeks, 91%; 24 weeks, 96%
Faldaprevir (BI 201335), deleobuvir (BI 207127), and ribavirin				
Zeuzem et al, 2013[66]	Phase IIb	362 gt1, tx-naïve	16, 28, or 40	SVR12: 69%
ABT-450/r, ABT-072, and ribavirin				
Lawitz et al, 2013[67]	Phase IIa	11 gt1 tx-naïve	12	SVR: 91%
Miracitabine (R7128), danoprevir (RG7227/ITMN-191), and ribavirin				
Gane et al, 2014[68]	Phase IIb	169 gt1, tx-naïve	12 or 24	SVR24: gt1a, 25%; gt1b, 64%
MK-5172, MK-8742, and ribavirin				
Hezode et al, 2014[69]	Phase II	156 gt1, tx-naïve	12	SVR12: 94—98%
Lawitz et al, 2014[70]	Phase II	254 cirrhotic or prior nonresponders	12 or 18	HCV RNA LLQ at week 4, 94—100%

gt1—gt6 = genotype 1—genotype 6; HCV = hepatitis C virus; IFN = interferon; LLQ = lower limit of quantification; SVR12 or SVR24 = sustained virological response at 12 or 24 weeks; tx-naïve = treatment-naïve.

once-daily daclatasvir and twice-daily asunaprevir for 24 weeks.[51] Eighty-seven percent of intolerant patients and 81% of prior nonresponders achieved SVR24. Ninety-one percent of cirrhotic patients achieved SVR24, compared to 84% of noncirrhotic patients. Thirteen percent of patients discontinued therapy because of nonresponse or because of adverse effects that typically included nasopharyngitis, elevated alanine aminotransferase (ALT) level, headache, diarrhea, or fever.

In a Phase IIa, open-label study, 101 patients with genotype 1b were randomly assigned to once-daily daclatasvir and once- or twice-daily asunaprevir with or without peginterferon and ribavirin for 24 weeks.[50] Seventy-eight percent of daclatasvir plus twice-daily asunaprevir patients achieved SVR12 and 95% of patients achieved SVR12 in quadruple therapy with peginterferon plus ribavirin. Common adverse events included headache, diarrhea, and weakness. Virological breakthrough was higher in patients with genotype 1a, which suggests that patients with genotype 1 should be treated according to subtype.

In the recent HALLMARK DUAL Phase III study, 305 treatment-naïve patients with genotype 1b were randomly assigned to treatment with 24 weeks of asunaprevir and daclatasvir or to 12 weeks of placebo, which was followed by entry into another study.[52] Four hundred and forty interferon-ineligible/intolerant patients or null/partial response patients were treated with asunaprevir and daclatasvir for 24 weeks. The SVR12 rates were similar in cirrhotic (85%) patients and noncirrhotic (84%) patients. The SVR12 rate was 90% in treatment-naïve patients and 82% in null/partial patients and in ineligible patients.

Asunaprevir, daclatasvir, and BMS-791325

In a Phase IIa open-label study (NCT01455090), 66 noncirrhotic treatment-naïve patients with genotype 1 were

treated for 12 weeks or 24 weeks with triple therapy that included asunaprevir, daclatasvir, and the NNI polymerase inhibitor BMS-791325.[53] Ninety-two percent of patients achieved SVR12, whereas one patient relapsed and two patients experienced viral breakthrough. Adverse events were mild and included headache, weakness, and gastrointestinal symptoms.

Daclatasvir and sofosbuvir

Daclatasvir was also paired with sofosbuvir with or without ribavirin in a recent once-daily, all-oral open-label study in 211 patients with genotypes 1, 2, or 3.[54] Ninety-eight percent of the patients with genotype 1 achieved SVR12, regardless of previous treatment failure with PIs. The SVR was slightly lower at 92% for patients with genotype 2 and 89% for patients with genotype 3. Adverse events included fatigue, headache, and nausea.

Ledipasvir and sofosbuvir

Several clinical trials have also paired sofosbuvir with the NS5A inhibitor ledipasvir. In a Phase III open-label study (NCT01701401), 865 treatment-naïve patients with genotype 1 were randomly assigned to fixed-dose, once-daily ledipasvir and sofosbuvir combination therapy with or without ribavirin for 12 weeks or 24 weeks.[55,56] The SVR12 rates ranged from 96% after 12 weeks of ledipasvir and sofosbuvir therapy to 99% in patients with 24 weeks of ledipasvir and sofosbuvir plus ribavirin triple therapy. In a related Phase III open-label study (NCT01768286), 440 patients with genotype 1 who failed to achieve SVR during previous peginterferon plus ribavirin therapy with or without telaprevir/boceprevir were randomly assigned to 12 weeks or 24 weeks of ledipasvir and sofosbuvir therapy with or without ribavirin.[55] Despite nonresponse to previous therapy and inclusion of patients with cirrhosis, the SVR12 rates ranged from 94% after 12 weeks of ledipasvir plus sofosbuvir dual therapy to 99% after 24 weeks of ledipasvir plus sofosbuvir with or without ribavirin. Common adverse events included fatigue, headache, and nausea in both studies.

Another Phase III, open-label study (NCT01851330) examined 8 weeks versus 12 weeks of ledipasvir plus sofosbuvir therapy with or without ribavirin in 647 treatment-naïve, noncirrhotic patients with genotype 1 infection.[57] Ninety-four percent of patients achieved SVR12 after 8 weeks of ledipasvir plus sofosbuvir therapy, compared to 95% after 12 weeks or 93% after 8 weeks with ribavirin. The authors found no additional benefit for the addition of ribavirin or for extending therapy from 8 weeks to12 weeks.

Sofosbuvir and ribavirin in genotypes 2 and genotype 3

Sofosbuvir has also been examined in single DAA therapy with ribavirin for patients with genotypes 2 or 3. Patients with genotypes 2 or 3 who are ineligible for or unresponsive to interferon therapy have few treatment options. In a pair of Phase III studies (NCT01542788 and NCT01604850) in patients with genotype 2 or genotype 3, 278 interferon-ineligible patients were randomly assigned to 12 weeks of therapy with ribavirin and either sofosbuvir or placebo, and 201 previously treated patients were assigned to 12 weeks or 16 weeks of sofosbuvir and ribavirin.[58] The SVR12 rate was 78% with sofosbuvir and 0% with placebo among interferon-ineligible patients. The SVR12 rate among previously treated patients was 50% with 12 weeks of therapy, compared to 73% after 16 weeks. Cirrhotic patients and patients with genotype 3 had lower response rates. The authors noted that the extension to 16 weeks of therapy significantly improved the response rates among previously treated patients with genotype 3.

Another recent study examined sofosbuvir and ribavirin in therapy in cirrhotic or previously treated patients with genotypes 2 or 3. Four hundred nineteen patients (21 patients with genotype 2 and 328 patients with genotype 3; 21% were cirrhotic and 58% were previously treated) were randomly assigned to receive 12 weeks of sofosbuvir and ribavirin or a placebo (NCT01682720).[59] However, in light of new information,[58] the study was unblinded and all patients with genotype 3 were treated with 24 weeks of sofosbuvir and ribavirin. Ninety-three percent of patients with genotype 2 and 85% of patients with genotype 3 achieved SVR12.

Sofosbuvir and simeprevir

Another recent combination includes sofosbuvir and the PI simeprevir. In the COSMOS study, 167 treatment-naïve or prior nonresponder patients were randomized into four groups treated with simeprevir and sofosbuvir with or without ribavirin for 12 weeks or 24 weeks.[60] Ninety-two percent of overall patients achieved SVR12, which included 90% among prior nonresponders with METAVIR scores F0—F2 and 92% among patients with METAVIR scores F3—F4. Fatigue, headache, and nausea were the most common adverse events, but severe adverse events were reported only in four patients.

ABT-450/r, dasabuvir (ABT-333), and ribavirin

In a Phase IIa open-label study (NCT01306617), 50 treatment-naïve patients or prior nonresponder patients with genotype 1 received 12 weeks of combination therapy with 150 mg or 250 mg of ritonovir-boosted ABT-450 (a PI), dasabuvir (a NNI polymerase inhibitor), and ribavirin.[61] Among treatment-naïve patients, 95% of the high-dose group and 93% of the low-dose group achieved SVR12. Forty-seven percent of the prior nonresponders achieved SVR12, but three patients relapsed and six patients experienced viral breakthrough. Adverse events included liver-enzyme abnormalities, fatigue, nausea, and rash.

ABT-450/r, ombitasvir, dasabuvir, and ribavirin

Several clinical trials have examined triple DAA combination therapy with ABT-450 with ritonavir (ABT-450/r, a protease inhibitor), ombitasvir (ABT-267, an NS5A inhibitor), and dasabuvir (ABT-333, a NNI polymerase inhibitor) with or without ribavirin. In a double-blind, placebo-controlled Phase III clinical trial (NCT01716585), 631 noncirrhotic treatment-naïve patients with genotype 1 were randomly assigned to 12 weeks of therapy with ABT-450/r,

ombitasvir, dasabuvir, and ribavirin or equivalent placebos.[62] The DAA group achieved a SVR12 rate of 96% (genotype 1a, 95%; genotype 1b, 98%), which was superior to the 78% SVR12 rate of a historical control group of patients treated with telaprevir, peginterferon, and ribavirin.

In two other Phase III clinical trials of treatment-naïve patients with genotype 1 (NCT01767116 and NCT01833533), 419 patients with genotype 1a, and 305 patients with genotype 1b were treated with 12 weeks of ABT-450/r, ombitasvir, dasabuvir, with or without ribavirin, or equivalent placebos.[63] A SVR12 was achieved in 99.5% of patients with genotype 1b and in 97% of patients with genotype 1a who received ribavirin, compared to 99% of patients with genotype 1b. In 90% of patients with genotype 1a, SVR12 was achieved without ribavirin. The rate of virologic failure was higher in patients with genotype 1a who did not receive ribavirin, but not in patients with genotype 1b.

Many studies have been performed on treatment-naïve noncirrhotic patients, although patients with cirrhosis or those who were nonresponsive to previous interferon therapy are likely to benefit from interferon-free therapies. In a Phase III trial (NCT01715415), 394 noncirrhotic patients with genotype 1 who experienced relapse, partial response, or null response under previous peginterferon plus ribavirin therapy were randomly assigned to 12 weeks of therapy with ABT-450/r, ombitasvir, dasabuvir, and ribavirin or their corresponding placebos.[64] Sustained virological response was achieved in 96% of patients in the active group (including 95% of prior relapsers), 100% of prior partial responders, and 95% of prior nonresponders.

To examine the effect of the interferon-free therapy in cirrhotic patients, 380 patients with genotype 1 with Child-Pugh class A compensated cirrhosis were treated for 12 weeks or 24 weeks with ABT-450/r, ombitasvir, dasabuvir, and ribavirin in an open-label Phase III.[65] Ninety-one percent of patients treated for 12 weeks achieved SVR, and 96% of patients treated for 24 weeks achieved SVR. Two percent of patients stopped treatment because of adverse events such as fatigue, headache, and nausea.

Faldaprevir (BI 201335), deleobuvir (BI 207127), and ribavirin

In a Phase IIb open-label clinical trial (NCT01132313) of faldaprevir (a PI) and deleobuvir (a NNI polymerase inhibitor), with or without ribavirin, 362 treatment-naïve patients with genotype 1 were randomly assigned to five treatment groups that differed with respect to treatment duration and deleobuvir dose.[66] The SVR12 ranged from 52% to 69% in the ribavirin groups, but was significantly higher than in the non-ribavirin group (39%). The SVR12 rates also varied with respect to the IL28B genotype and were higher for HCV genotype 1b than for HCV genotype 1a. However, the pharmaceutical company Boehringer Ingelheim (Ingelheim, Germany) has discontinued development of the drug because of insufficient efficacy in follow-up studies.

ABT-450/r, ABT-072, and ribavirin

In a Phase IIa open-label single-arm study, 11 treatment-naïve patients with genotype 1 were treated with ritonavir-boosted ABT-450 (PI), ABT-072 (NNI polymerase inhibitor), and ribavirin for 12 weeks.[67] Ninety-one percent of patients achieved a SVR, although one patient relapsed 36 weeks after the end of treatment. Adverse events included headache, fatigue, and nausea.

Mericitabine (R7128), danoprevir (RG7227/ITMN-191), and ribavirin

In a Phase IIb follow-on study (NCT01278134), 169 treatment-naïve patients with genotype 1 were treated with mericitabine and ritonavir-boosted danoprevir with ribavirin or placebo for 12 weeks or 24 weeks.[68] Relapse rates were high with the 12-week regimen and ribavirin-free regimen, primarily because of danoprevir-resistant mutations. Thirty-seven percent of 24-week patients achieved SVR24, but patients with genotype 1a had much poorer response (25%) than patients with genotype 1b (64%). The authors concluded that the SVR rates were poor, although the combination therapy was safe and well tolerated.

MK-5172, MK-8742, and ribavirin

Results of the Phase II the Study of the Combination Regimen MK-5172 and MK-8742 ± Ribavirin in Participants with Chronic Hepatitis C (C-WORTHy) trial were presented at the 49th Annual Meeting of the European Association for the Study of the Liver (EASL 2014). In Part A, 65 noncirrhotic, treatment-naïve patients with genotype 1 were randomized into three treatment arms and treated for 12 weeks with 20 mg or 50 mg of once-daily MK-5172 (a PI) and MK-8742 (a NS5A inhibitor) with or without ribavirin.[69] In Part B, 91 patients were assigned to three treatment arms with 50 mg MK-5172/MK-8742/ribavirin for 8 weeks or 12 weeks. Hepatitis C virus RNA was below the lower limit of quantification (LLQ) at Week 4 in all patients. Ninety-eight percent of patients treated with the MK-5172/MK-8742 achieved SVR12, whereas 94% of patients treated with MK-5172/MK-8742/ribavirin achieved SVR12. The most common adverse events included fatigue, headache, and nausea. In another trial of the C-WORTHy study, 254 patients with cirrhotic or previous null response were randomly assigned to treatment with 12 weeks or 18 weeks of MK-5172/MK-8742 therapy with or without ribavirin.[70] Patients showed rapid suppression of the virus, with 59—73% of patients reaching the HCV RNA LLQ by Week 2 and 94—100% of patients reaching the LLQ at Week 4. The most common adverse events were fatigue, headache, and nausea.

Conclusion

The large number of DAAs currently under development and the encouraging results in clinical trials are cause for optimism in successfully treating HCV. However, as additional DAAs are approved for clinical use, clinicians may face complex choices and treatment guidelines. This will allow greater potential for personalized therapies and require increased need for resistance monitoring and stopping rules. Future clinical trials will examine new drugs and are likely to consider optimal DAA combinations and

evaluate the ongoing need for ribavirin, balancing safety, efficacy, and barriers to resistance. Upcoming clinical trials should also address remaining difficult-to-treat patient populations with unmet needs, including pre- and post-liver transplantation patients and uremic or HCV/HIV coinfected patients. In principle, all patients could be treated with interferon-free DAA combination therapies; however, in developing countries or in the event of strong resistance or other complications, interferon and ribavirin are likely to continue to have an important role.[71] In this scenario, patient screening may help to identify the most effective and cost-effective treatment approaches in situations of limited resources.

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